PB85-147205

Measurement of Ventilatory Frequency in Unrestrained Rodents Using Microwave Radiation

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

1984

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TECHNICAL REPORT DATA
(Please read Instructions on the reverse before complet

1. REPORT NO.	2.	3.
EPA-600/J-84-176		PB85-147205

4. TITLE AND SUBTITLE	5. REPORT DATE
Measurement of Ventilatory Frequency in Unrestrained Rodents Using Microwave Radiation (Journal Version)	1984
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Christopher J. Gordon and Joseph S. Ali	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
U. S. Environmental Protection Agency Health Effects Research Lab Research Triangle Park, NC 27711	AC6FIA
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
same as #9	14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES

Respiration Physiology (1984) 56, 73-79

16. ABSTRACT

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Breathing frequency — Mouse
Microwave technique — Pneumography

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
RELEASE TO PUBLIC	unclassified	8
	20. SECURITY CLASS (This page) unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITI

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INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

EPA-600/J-84-176
JOURNAL ARTICLE

Respiration Physiology (1984) **56**, 73–79
Elsevier

MEASUREMENT OF VENTILATORY FREQUENCY IN UNRESTRAINED RODENTS USING MICROWAVE RADIATION

CHRISTOPHER J. GORDON and JOSEPH S. ALI

Experimental Biology Division, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711, U.S.A.

Abstract. A novel technique for remote determination of breathing frequency in unrestrained rodents using microwave radiation is described. Single mice were placed inside a rectangular waveguide operating at 2450 MHz. Because mice efficiently absorb radio frequency energy at 2450 MHz, any change in their absorption, as occurs rhythmically with breathing, can be detected by monitoring the changes in power transmitted through the waveguide. When the volume of the mouse increases during inspiration, transmitted power through the waveguide momentarily decreases – during expiration the reverse takes place. By differential amplification of the analog outpout of the power meter used to monitor transmitted power, breathing frequency can be easily recorded on conventional recording equipment. The microwave technique has a definite advantage over other methods in that breathing frequency can be remotely monitored without the need to attach wire leads or surgically implant telemetry probes. This greatly reduces unnecessary stress to the animal. By use of larger waveguides the principle of this technique may be applied to larger species.

Breathing frequency Mouse
Microwave technique Pneumography

The ventilatory frequency of laboratory mammals has conventionally been determinded by (i) visual counting of the chest movements, (ii) recording the change in transthoracic impedance by means of electrodes attached to the chest or (iii) monitoring chest volume with plethysmograph transducers. The first method cannot be used in small rodents where the ventilatory frequency is so high that the chest movements cannot be counted visually. The latter two methods can add a variety of artifacts to the measurement of ventilatory frequency. Attachment of leads to the animal creates anxiety which may result in higher than normal breathing frequencies. In addition, for unrestrained animals the attached wires may become tangled and impede free movement. Surgically-implanted telemetry capsules provide the only method for measuring breathing frequency in unrestrained animals

Accepted for publication 28 December 1983

without use of attached leads. However, this method may create problems in situations where one wishes to avoid surgical traumatization of the animal.

It is the purpose of this paper to demonstrate a technique for measuring ventilatory frequency in unrestrained animals using radio frequency (RF) electromagnetic waves. The method relies on a simple principle that when an animal inhales and exhales its absorption of RF energy changes concomitantly. By monitoring the rhythmic fluctuation in transmitted RF power, ventilatory frequency can be continuously recorded in unrestrained animals.

Materials and methods

Equipment. A schematic of the RF exposure facility along with the equipment for monitoring ventilatory frequency is shown in fig. 1. The frequency of RF radiation used in this study was 2450 MHz (*i.e.*, in the microwave region of the RF spectrum). The microwave generator, which was designed and built by laboratory

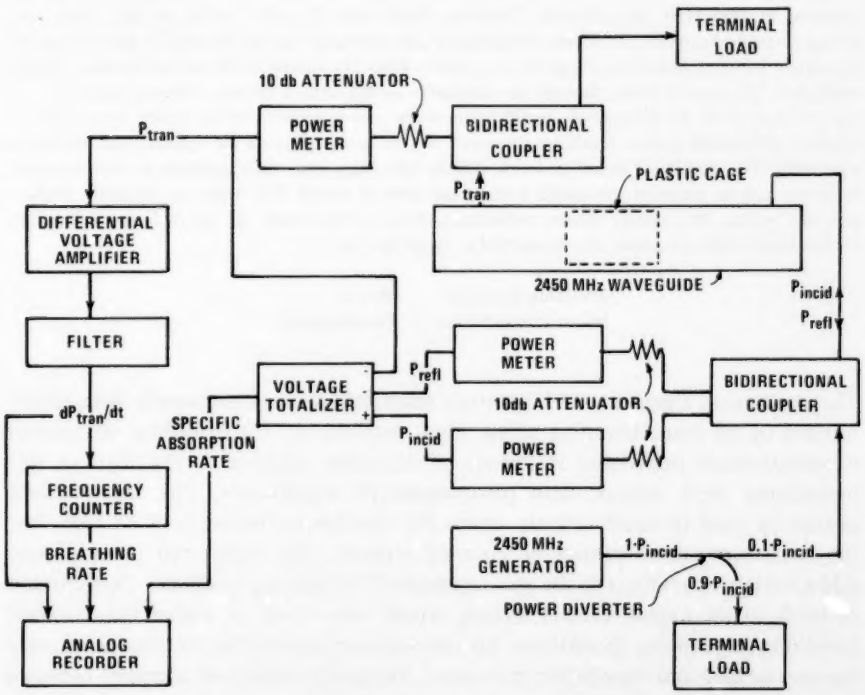

Fig. 1. Diagram of 2450 MHz microwave exposure system and ancillary equipment for monitoring ventilatory frequency in mice. Not shown are holes in waveguide for continuous circulation of air. See text for further details.

staff, is capable of providing up to 200 W of output power. However, at relatively low powers (<30 W) the signal output contains an unwanted 60 Hz modulation component. Hence the generator is normally maintained at a power output above 30 W. For low power operation, a diverter circuit shunts 90% of the generator power to a terminal load while the remaining 10% is fed to a Narda Model 3022 bidirectional coupler. The bidirectional coupler, in conjunction with two Hewlett-Packard 435A power meters, is used to monitor incident power (P_{inc}) entering the waveguide and reflected power (P_{rfl}) emanating from the waveguide back to the generator. Power from the coupler to the power meter is attenuated by a factor of 1/1000 in order to prevent overload to the power meters. Transmitted RF power not absorbed by the animal (P_{tran}) is monitored by another bidirectional coupler and power meter located at the other end of the waveguide. The transmitted power *per se* is absorbed by another terminal load on the waveguide.

The DC analog output derived from the power meter used to monitor transmitted power (0–1 V, full scale) is amplified with a high gain, differential amplifier (Beckman 9853A voltage coupler having a low frequency response of 0.16 Hz). The output from the coupler's preamplifier was fed to a Beckman 411 N amplifier with a high frequency cutoff of 30 Hz.

Using differential amplification the rhythmic changes in transmitted power, associated with ventilatory-induced movements of the animal's thorax, were readily recorded on a analog chart recorder. The net voltage sensitivity of the recording system was set at 0.05 to 0.1 mV/mm chart deflection. Differentially amplified transmitted power data (*i.e.*, dP_{tran}/dt) were also passed through a frequency counter to give a continuous recording of ventilatory frequency.

Procedure. A circular Plexiglas cage containing a single mouse was placed inside a section of WR 430 standard waveguide having internal dimensions of height, 5.8 by width, 11.2 cm (there is no limit on the length of waveguide required). Plexiglas is virtually transparent to 2450-MHz RF radiation. A middle section of the waveguide of length 20 cm which contained the Plexiglas cage was immersed in a water bath maintained at 31 °C. Water immersion allowed for isothermal control of the air and wall temperatures. Fresh air was continually circulated through the interior of the waveguide. After a 1 h accomodation period the microwave generator was activated at various incident powers while ventilatory frequency and the rate of absorption of microwave energy were continuously recorded. Specific absorption rate (SAR) was then computed using the formula: $SAR = P_{inc} - P_{rfl} - P_{tran}$/body wt. Generally, mice with a body weight of 25 to 35 g, as was used in this study, will absorb between 50 to 80% of the incident power. Power absorption is dependent on the orientation of the mouse in the waveguide. Absorption is maximal when the mouse is oriented parallel with respect to the longitudinal axis of the waveguide. For further details on measuring SAR, see Gordon (1982).

To verify that the rhythmic changes in transmitted power were a result of

breathing, freshly killed mice were intubated with a polyethylene cannula which was connected to a small animal respirator. The animals were artificially ventilated inside the waveguide during microwave exposure. By noting the phase of the respiratory cycle with the recording of dP_{tran}/dt, it was possible to correlate the simulated breathing movements with changes in transmitted power.

Results and Discussion

Examples of breathing patterns of mice exposed to incident powers of 0.38 and 0.76 W are shown in fig. 2. The incident power levels used resulted in whole-body SAR's of 8.9 and 18.2 W/kg, respectively. At the lower power setting the ventilatory frequency was approximately 100 b/m which is lower than what one could expect for a mouse (*e.g.* ≈140 b/m; see Guyton, 1947; Stahl, 1967). At the higher power level there was evidence of heat stress from the absorbed RF radiation since ventilatory frequency was over 200 b/m. Any movement by the animal (see arrows in fig. 2) resulted in movement artifacts on the recording of dP_{tran}/dt and momentarily prevented recording of breathing activity. Shifts in animal posture and orientation relative to the microwave field result in larger changes in absorbed RF energy

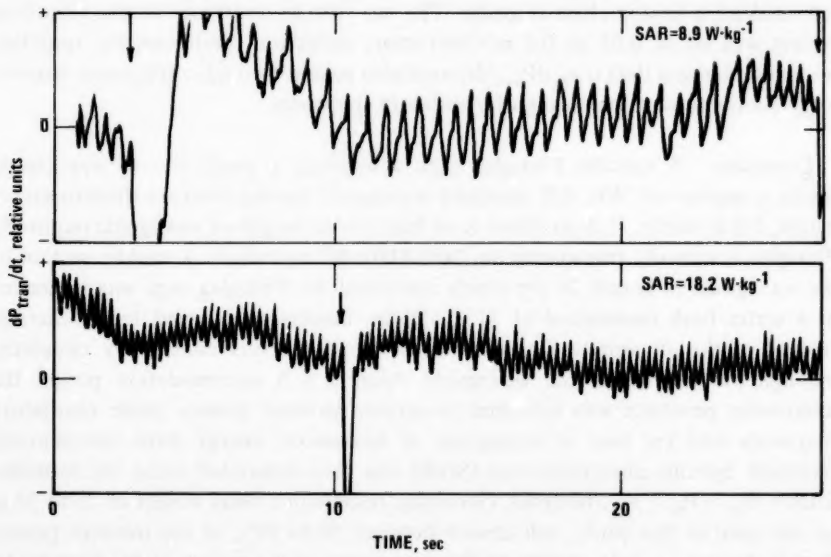

Fig. 2. Examples of ventilatory frequency records in unrestrained mice exposed to 2450 MHz at incident powers of 0.38 (upper panel) and 0.76 W (lower panel). Increase in dP_{tran}/dt is associated with expiration and decrease is associated with inspiration. Note elevated breathing rate at higher specific absorption rate (SAR) which is due to the excessive thermal loading from microwave exposure. Arrows indicate movement by mouse in the waveguide.

when compared to the changes due to breathing. Hence, breathing activity can be monitored only in those animals that remain relatively quiescent.

Measurements made in artificially ventilated dead mice demonstrated that the downward deflection in dP_{tran}/dt was caused by inspiration and the upward deflection by expiration. That is, with inspiration the volume of the animal enlarges resulting in an increase in absorbed power and a drop in P_{tran}. Since the differential of P_{tran} with time is measured, we could not measure absolute volume of the lungs (*e.g.*, tidal volume) with the existing equipment. It may be possible, with additional electronic processing, to derive lung volume from the measurement of P_{tran}.

The microwave technique has been used to measure ventilatory frequency in unrestrained mice at incident powers as low as 0.08 W which is equivalent to an SAR of approximately 2.0 W/kg (Gordon, 1983). It is difficult to monitor breathing activity at lower incident powers owing to poor signal-to-noise ratios in the power meters and recording equipment. Normally the power meters are set at 1 or 3 mW full scale. At lower meter sensitivities the breathing patterns are detectable but are somewhat distorted.

It is important to consider if measuring ventilatory frequency with the microwave method causes heat stress in the animals. Excess heat stress would elevate ventilatory frequency above baseline levels. However, at 22 °C an SAR of approximately 22 W/kg is required to cause a significant elevation in rectal temperature of mice (Berman *et al.*, 1978). There is a inverse correlation between ambient temperature and threshold SAR for elevating ventilatory frequency (Gordon and Long, 1984). For example, the threshold SAR for increasing breathing frequency in mice is 10 W/kg at 30 °C and 50 W/kg at 20 °C. Therefore, this method is usable to measure baseline ventilatory frequency provided that the combined effects of ambient temperature and microwave exposure does not lead to thermal-induced increases in ventilatory activity. It has been observed (Gordon, 1983) that when mice are initially exposed to thermally-stressful SAR's breathing activity during the first 15–20 sec of exposure is at the same low level as that seen in non-stressed animals. Hence, in cases where low SAR's are not practical, baseline ventilatory frequency may still be measured at relatively high SAR's by observing the first few seconds of breathing activity at the onset of RF exposure.

Optimizing the sensitivity of this technique requires that the animal being observed efficiently absorb a large part of the incident RF energy. Maximum absorption is obtained when conditions of interaction are at or near resonant absorption. This condition is dependent on a number of factors (Durney *et al.*, 1978; Guy, 1971) but requires, in general, that the wavelength of the incident radiation be comparable with the size of the animal being observed. For example, resonant absorption in the mouse takes place near 2450 MHz, whereas for an adult human the resonant frequency is reduced to 80 MHz. It is important, therefore, to properly select the optimal RF frequency of operation for the particular animal being studied.

The waveguide used in this study operates over the frequency range of 2200–3300 MHz. Operation at lower RF frequencies requires a waveguide of larger

cross sectional area. This is a beneficial relationship from the standpoint that larger animals are characterized by a lower resonant frequency: operation at this frequency requires a waveguide large enough to accomodate the animal. For example, a larger rectangular waveguide (38 × 19 cm) operating at a frequency range of 400–800 MHz is currently being used in our laboratory. The waveguide is large enough to accomodate a rat or guinea pig, animals that have near-resonant frequencies of approximately 490–750 MHz (Durney *et al.*, 1978). Future studies in this laboratory will be directed toward assessing the feasibility of measuring ventilatory frequency in other mammals using larger waveguides at lower RF frequencies.

Lin (1975) described a noninvasive method of measuring ventilatory frequency by exposing animals to 10 GHz RF radiation. However, his method differs substantially from that of the present study. In the method of Lin, the change in reflected energy resulting from chest movements is compared with the level of incident power to give a remote monitoring of ventilatory frequency. Thus the technique of Lin relies on the principle of change in reflectance to 10 GHz RF energy whereas the present study measured the change in transmitted 2.45 GHz energy. One advantage of the waveguide method is that SAR can be monitored directly.

To summarize, this paper has demonstrated a novel method for remotely measuring ventilatory frequency in unanesthetized and unrestrained mice using microwave radiation. The method is unique in that measurements can be made on animals without surgical intervention (*i.e.*, for implanting telemetry capsules) and without attaching wires to the animal. Measuring ventilatory frequency without use of the above described interventions may be of value to an array of physiological studies.

Acknowledgements

I thank the following people who provided critiques of the manuscript: Dr. C. Weil, Dr. J. H. Ferguson, A. Rezvani and S. Trautwein.

This paper has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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